1	Traditional water structures in villages support amphibian populations within a protected
2	landscape
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Abstract

Amphibians are among the most globally threatened vertebrates, with habitat loss and degradation being the primary drivers of their decline. While natural wetlands are essential for amphibian survival, artificial habitats can also play a significant role as refuges, especially in human-altered landscapes. This study examines the role of artificial waterbodies in supporting amphibian populations within villages and human-disturbed areas of Peneda-Gerês National Park (PNPG) in northern Portugal, a unique protected area recognized for its rich natural and cultural heritage. We conducted surveys across 162 natural (ponds, streams, stream pockets, caves) and artificial (tanks, drains, fountains, cave-like structures), waterbodies, to assess species richness, abundance, and breeding activity in human-altered landscapes within PNG. A total of ten amphibian species were observed, with natural waterbodies showing higher species richness and occupancy rates. The Iberian frog (Rana iberica) was the most abundant species, found primarily in natural habitats, where it bred exclusively. Although the fire salamander (Salamandra salamandra) was also most common in natural waterbodies, it bred across a wide range of both natural and artificial waterbodies. In contrast, the endemic Bosca's newt (Lissotriton boscai) and the marbled newt (*Triturus marmoratus*) were more prevalent in artificial waterbodies,

29 particularly in historic water tanks. These water tanks, traditionally used for laundry and water 30 storage in local villages, were crucial for these amphibians, with approximately two-thirds 31 occupied and over a quarter serving as breeding sites for four different species—supporting more 32 species than all the natural waterbodies combined. These findings emphasize the need to 33 integrate the conservation of both natural and artificial aquatic habitats to sustain amphibian 34 biodiversity, particularly in human-altered landscapes like PNPG. As climate change diminishes 35 natural breeding sites, artificial waterbodies can offer crucial refuges that complement natural 36 habitats, playing a vital role in protecting both biodiversity and the region's cultural heritage. 37 Key Words: Amphibians; Artificial habitats, Bosca's newt (Lissotriton boscai); Cultural Heritage; Drainage Systems (Open Channels and Drains); Fire salamander (Salamandra 38 39 salamandra); Iberia; Marbled newt (Triturus marmoratus); Portugal; Peneda-Gerês National 40 Park; Rana iberica (Iberian frog); Fire salamander (Salamandra salamandra); Water Tanks

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42 Introduction

43 Amphibians are the most globally threatened vertebrate group, with more than 40% of all 44 species at risk of extinction, primarily driven by habitat loss and degradation, emerging 45 infectious diseases, and the effects of climate change (Stuart et al. 2004, Luedtke et al. 2023). 46 Habitat loss in particular poses the most critical threat impacting 93% of endangered amphibian 47 species (Luedtke et al. 2023). The loss and alteration of both aquatic breeding grounds and 48 terrestrial habitats plays a significant role by disrupting the complex biphasic life cycles typical 49 of most amphibians (Cushman 2006, Becker et al. 2007). Their highly permeable skin also 50 makes them particularly vulnerable to environmental changes, making them sensitive to 51 fluctuations in moisture, temperature, and pollutant exposure in both aquatic and terrestrial 52 environments (Alford and Richards 1999). However, the impact of habitat changes on amphibian 53 populations varies across species and habitats (Hamer and McDonnell 2008, Pyron 2018, Valdez 54 et al. 2021). While pristine wetlands and forests provide ideal conditions, some artificial habitats 55 can offer supplemental refuge when natural areas are lost or degraded. Recent studies show that roughly one-third of the world's amphibian species use artificial 56

57 habitats to some extent, even occupying heavily altered environments (Warren and Büttner 2008, 58 Valdez et al. 2015, 2021). Although not a substitute for natural habitats, constructed waterbodies 59 like drainage ditches, rice paddies, agricultural ponds, and wastewater treatment ponds can 60 provide vital alternative aquatic breeding grounds, helping to support and sustain populations of 61 threatened amphibian species when natural habitats become scarce or degraded (Knutson et al. 62 2004, Brand and Snodgrass 2010, Valdez et al. 2015, Boissinot et al. 2019, Caballero-Díaz et al. 63 2020, Yu et al. 2022, Conan et al. 2023, Romano et al. 2023). Additionally, terrestrial habitats 64 like plantations, pastures, gardens, and urban greenspaces can serve as habitats when forests

65 become fragmented or degraded (Hartel 2004, Manenti et al. 2013, Holzer 2014, Valdez et al. 2021, Yu et al. 2022). Nevertheless, while some artificial habitats can support certain amphibian 66 67 species, many others are less beneficial due to limitations such as altered hydrology, ecological traps, pollution, and invasive species, which can lead to lower survival rates and reduced 68 69 biodiversity compared to natural areas (Knutson et al. 2004, Hamer and McDonnell 2008, 70 Gordon et al. 2009, Price et al. 2011, Cordier et al. 2021, Băncilă et al. 2023). Determining 71 whether individual artificial habitats support or threaten particular amphibian populations is key 72 to evaluating their long-term conservation value, especially in regions with a legacy of extensive 73 anthropogenic landscape alteration. In Europe, for example, approximately 80% of landscapes have been extensively 74 75 transformed over the past centuries due to agricultural intensification, urbanization, and 76 infrastructure expansion (Pedroli and Meiner 2017, European Environment Agency 2023). These 77 changes have led to the loss of over 50% of wetlands in many European countries due to the

draining of floodplains and peatlands for agriculture and urbanization (Fluet-Chouinard et al.
2023). Meanwhile, since the 1990s, the expansion of artificial land has accelerated more than
any other land cover type, driven by ongoing urbanization and infrastructure construction
(Pedroli and Meiner 2017). Nevertheless, some artificial habitats such as stormwater ponds,
highway drainage systems, and fish farms have been found to partly mitigate the impact of
natural habitat loss for some species in certain areas (Kloskowski 2010, Le Viol et al. 2012,
Conan et al. 2023).

In the drought-prone Mediterranean climate of the Iberian Peninsula, encompassing
Spain and Portugal, artificial water bodies may be especially valuable for amphibian species.
Evidence suggests that structures such as irrigation canals, farm ponds, water tanks, ditches, and

88 abandoned quarries can serve as habitats, providing critical network connectivity for dispersal 89 and additional breeding habitats for many amphibian populations in this water-scarce and heavily altered region (Garcia-Gonzalez and Garcia-Vazquez 2011, Ferreira and Beja 2013, Galvez et al. 90 91 2018, Caballero-Díaz et al. 2020, 2022, Gutiérrez-Rodríguez et al. 2023). Understanding 92 amphibian use of artificial habitats is vital to support populations now reliant on these man-made 93 habitats, especially in the Iberian region, which contains the highest concentration of endemic 94 and threatened amphibian species in Europe (Temple and Cox 2009, Luedtke et al. 2023). 95 While many studies have explored the importance of specific artificial habitats for

96 amphibians, there appears to be a lack of studies on the use of these habitats within protected areas. Peneda-Gerês National Park (PNPG) in northern Portugal, the oldest protected area and 97 98 the only national park in the country, offers an ideal setting to investigate amphibian use of 99 artificial habitats within a protected area (Soares et al. 2005). Established in 1971 and part of the 100 "Natura 2000" network of European priority conservation areas, PNPG is situated at the 101 crossroads of Euro-Siberian and Mediterranean zones, creating a unique climatic transition from 102 Atlantic to Mediterranean conditions (Soares and Brito 2007). This blending of two distinct 103 bioclimatic regions enables the park to serve as a biodiversity hotspot, hosting thirteen 104 amphibian species and four Iberian endemics that thrive in its pristine montane streams, rivers, 105 and ponds (Soares et al. 2005). However, the park also encompasses traditional mountain 106 villages, home to centuries-old artificial waterbodies like historic stone fountains, communal 107 laundry and water tanks, and drainage channels that were once vital to traditional village life 108 (Soares and Brito 2007, Cabral et al. 2017, Simões et al. 2019, Martins 2022). These historically 109 significant structures not only serve as cultural landmarks but also present a valuable opportunity 110 to explore their potential as biodiversity refuges for amphibians within this unique protected

area. Understanding the role of artificial habitats in PNPG is crucial for managing and protecting
amphibian populations within this ecologically rich landscape shaped by natural and cultural
elements.

114 In this study, we investigate the role of artificial waterbodies in supporting amphibian 115 populations within Peneda-Gerês National Park (PNPG), focusing on villages and other human-116 altered areas within its protected landscape. We compare amphibian species richness, abundance, 117 and breeding activity between artificial waterbodies (tanks, drains, fountains, and cave-like 118 structures) and natural habitats (ponds, streams, stream pockets, and caves). Additionally, we use 119 Principal Component Analysis (PCA) to examine the differences and similarities in habitat 120 characteristics across the various waterbody types. This study aims to understand the ecological 121 significance of artificial waterbodies in supporting amphibian populations within this unique 122 protected area, where natural and cultural heritage intersect.

123 Methods

124 <u>Study area</u>

The study was conducted over two survey periods: May 17-21, 2023, and May 6-12,
2024. It covered 11 villages and human-disturbed areas within Peneda-Gerês National Park in
northern Portugal, including Alcobaça, Assureira, Barreiro, Castro Laboreiro, Couscadas, Dorna,
Lamas de Mouro, Mareco, Pousios, Ribeiro de Beixo, and Ribeiro de Cima. In total, 162
waterbodies were surveyed, consisting of 68 artificial and 94 natural waterbodies (Appendix S1:
Figure S1).

132 We categorized the various waterbodies into natural and artificial types (Figure 1). For 133 natural habitats, we identified four categories: ponds, streams, stream pockets, and caves. Ponds 134 (Figure 1a) are small natural standing bodies of freshwater, while streams (Figure 1b) are small, 135 shallow, naturally flowing bodies of water typically originating from springs or rainfall. Stream 136 pockets (Figure 1c) are localized areas within deeper and wider stream systems where water flow 137 is more concentrated or pooled. Caves (Figure 1d) refer to naturally formed hollow spaces or 138 chambers within rock formations that contain bodies of water. Within the artificial category, we 139 identified four types of waterbodies: tanks, drains, fountains, and cave-like structures. Tanks 140 (Figure 1e) are artificial containers historically used for storing water, often for laundry or 141 troughs. Drains (Figure 1f) are man-made structures typically located at ground level, such as 142 open drainage channels, designed to redirect excess rainwater and runoff, preventing water 143 accumulation in village streets and agricultural areas. Fountains (Figure 1g) are ornamental 144 features with flowing water situated at higher elevations. Cave-like structures (Figure 1h) are 145 artificial, enclosed spaces that mimic the appearance and environment of natural water-146 containing caves.



Figure 1. Types of waterbodies surveyed within Peneda-Gerês National Park. Natural
waterbodies include (a) ponds, (b) streams, (c) stream pockets, and (d) caves. Artificial
waterbodies are represented by (e) tanks, (f) drains, (g) fountains, and (h) cave-like structures.

151 <u>Survey sampling</u>

Amphibian sampling was conducted through systematic nocturnal surveys typically between 20:30 and 02:00. We searched waterbodies by walking along their perimeters, using visual encounter surveys (VES) to observe amphibians. Additionally, auditory sampling was performed to record species based on calls. Dip-net sweeps in a figure-8 motion were used to collect amphibian larvae and adults for counting and species identification. The presence of larvae was taken as evidence of breeding at the waterbody. We measured environmental variables, including waterbody dimensions, depth, turbidity, temperature, pH, water flow. Additionally, we estimated the percentage cover of habitat features including rocks, mud, leaflitter, and aquatic vegetation.

161 *Statistical analyses*

162 We used R version 4.2.2 to analyze differences in amphibian occupancy, abundance, and 163 breeding across species, as well as between artificial and natural waterbody types. A Chi-square 164 test of independence was employed to examine the relationship between species, waterbody 165 types, and occupancy status (occupied vs. unoccupied sites). Mean abundance differences across 166 species and waterbody types were assessed using a one-way ANOVA, with post-hoc tests to 167 identify significant pairwise differences. Welch's two-sample t-test was used to compare mean 168 amphibian abundance between artificial and natural habitats. Additionally, Chi-square tests were 169 performed to explore associations between species, waterbody types, and breeding activity.

170 We conducted a Principal Component Analysis (PCA) using the FactoMineR package in 171 R to explore the relationship between waterbody characteristics. Continuous variables were 172 standardized for comparability, and categorical variables were converted into dummy variables. 173 Rows with missing data were removed to create a clean dataset. After an initial PCA, we applied 174 a contribution threshold of 5% to focus on the most significant variables contributing to the 175 variation in the first two principal components. Variables exceeding this threshold were retained, 176 and the PCA was rerun using the reduced dataset. The results were visualized with the factoextra 177 package, using a gradient color scale to highlight the contribution of each variable and identify 178 the key characteristics differentiating the waterbodies.

179 **Results**

180 <u>Species richness</u>

181 We found ten amphibian species within our study area, comprising six frog species 182 (Order: Anura) and four salamander species (Order: Urodela). Among the frogs, we recorded the 183 Iberian frog (Rana iberica), Perez's frog (Pelophylax perezi), Spiny toad (Bufo spinosus), 184 Common midwife toad (Alytes obstetricans), Natterjack toad (Epidalea calamita), and Iberian 185 painted frog (Discoglossus galganoi). The salamander species included the Fire salamander 186 (Salamandra salamandra) and three newt species within the subfamily Pleurodelinae: Marbled 187 newt (Triturus marmoratus) and Bosca's newt (Lissotriton boscai), along with the Iberian ribbed 188 newt (Chioglossa lusitanica).

189 Natural waterbodies had the highest species richness, with nine out of the ten amphibian 190 species observed, whereas artificial waterbodies hosted only seven species (Appendix S1: Figure 191 S1). Discoglossus galganoi, Epidalea calamita, and Chioglossa lusitanica were exclusively 192 found in natural habitats, while Alytes obstetricans were only observed in artificial water bodies 193 (Appendix S1: Figure S2). However, we also heard the midwife toad's call (*Alytes obstetricans*) 194 in nearby natural habitats, suggesting its presence there despite not being visually confirmed. 195 Amphibian species richness also varied across different waterbody types (Appendix S1: Figure 196 S2). Within natural waterbodies, stream pockets, ponds, and streams each had six species, while caves had four species (Appendix S1: Figure S2). In contrast, drains had the highest richness 197 198 among artificial and all waterbodies, with seven species, followed closely by tanks with six 199 species (Appendix S1: Figure S2). Artificial caves and fountains were less diverse, containing 200 only two and one species, respectively (Appendix S1: Figure S2).

202	There were significant differences in mean abundance across different waterbody types
203	and species. ANOVA results indicated significant effects for the different waterbody types (F =
204	20.17, $p = 0.048$), species (F = 61.40, $p = 0.0161$), and their interaction (F = 31.43, $p = 0.0313$).
205	Although natural waterbodies exhibited a higher mean abundance (4.65) compared to artificial
206	sites (3.32), it was not significant (t = -1.612, df = 124.52, $p = 0.054$). Nevertheless, this slightly
207	higher abundance observed may be largely attributed to the abundance of <i>R. iberica</i> , the most
208	abundant species with 337 individuals observed, representing 61.27% of all amphibians
209	recorded. The vast majority (90.8%) were found in natural waterbodies, particularly in stream
210	pockets, ponds, and streams (Figure 2). L. boscai was the second most abundant species (56
211	individuals) and predominantly found in artificial waterbodies (78.6% of observations), with the
212	highest abundance in tanks (Figure 2). S. salamandra was the third most common (55
213	individuals) and mainly observed in natural habitats (87.3% of observations), especially in
214	stream pockets (Figure 2). P. perezi had 43 individuals recorded and was mainly (62.8%) found
215	in natural waterbodies, especially ponds, but also in artificial tanks (Figure 2). T. marmoratus
216	had 42 individuals and was mostly observed (71.4% of observations) in artificial sites,
217	particularly in tanks (Figure 2). The remaining species were much less abundant, but B. spinosus,
218	and A. obstetricans were mostly found in artificial habitats (drains and tanks) while E. calamita,
219	D. galganoi, C. lusitanica were found only in natural habitats (Figure 2).

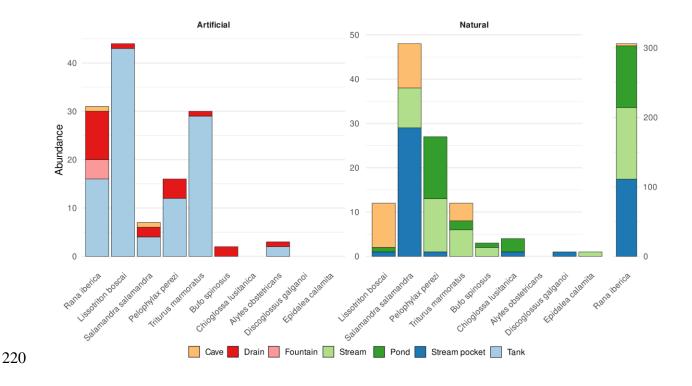
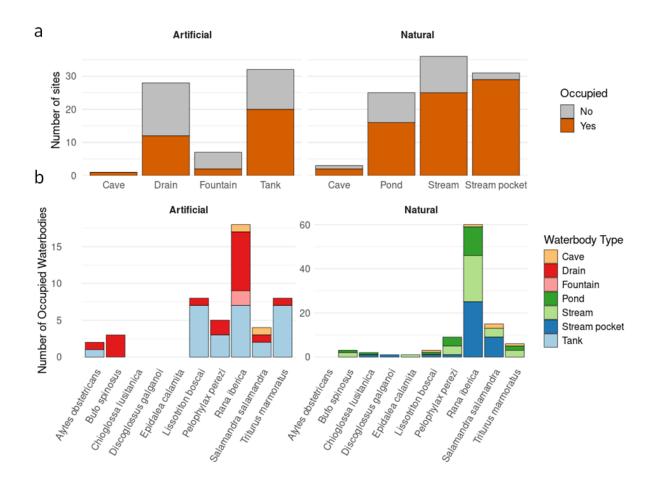


Figure 2. Stacked bar plot showing the abundance of amphibian species observed in different
 types of artificial and natural waterbodies in Peneda National Park.

223 Site occupancy

There was a significant difference in occupancy rates between artificial and natural waterbodies, with 35 (51.5%) artificial sites and 72 (76.6%) natural sites occupied with at least one species ($X^2 = 10.015$, df = 1, p = 0.0016). Over the two survey periods, all of the different types of natural waterbodies had most (at least 50%) of their sites occupied at least once. In contrast, among artificial waterbodies, only tanks had the majority of their sites occupied across the study area. Additionally, only six artificial (8.82%) and 16 natural (17.02%) waterbodies were occupied in both years.

231 The occupancy rates for waterbody types reveal significant differences between all the 232 artificial and natural waterbody types (Fisher's Exact Test: p < 0.001). Natural waterbodies 233 exhibited generally higher occupancy rates, with stream pockets showing the highest occupancy 234 at 96.6%, followed by streams (69.4%), ponds (64%), and caves (66.7%) (Figure 3a). In contrast, 235 among artificial waterbodies, occupancy was highest in tanks (62.5%), followed by drains 236 (42.9%), fountains (28.6%), and caves (100%), although the latter was based on a single site. 237 Occupancy also rates differed significantly among species ($X^2 = 357.9$, p < 0.001). R. 238 *iberica* was by far the most widespread species found in 78, or nearly half (48.1%) of all 239 waterbodies surveyed, with most occurring in natural waterbodies (60 sites or 63.8% of all 240 natural sites) compared to artificial ones (18 sites or 26.5% of all artificial waterbodies) (Figure 241 3b). S. salamandra was present in 19 sites (11.7% of all waterbodies), predominantly in natural 242 waterbodies (15 sites or 16.0% of all natural sites) (Figure 3b). T. marmoratus and P. perezi were 243 each found in 8.6% of all waterbodies, with T. marmoratus more common in artificial 244 waterbodies (eight sites or 11.8% of artificial waterbodies) and P. perezi in natural ones (nine 245 sites, or 9.6% of natural waterbodies) (Figure 3b). L. boscai was recorded at eleven waterbodies, 246 mostly in artificial waterbodies (8 sites or 11.8% of artificial waterbodies) (Figure 3b).



247

Figure 3: Total number of occupied and unoccupied waterbodies across different artificial and
natural types (a) and species-specific occupancy in artificial and natural waterbodies (b) in
Peneda-Gerês National Park.

251 <u>Reproduction</u>

Breeding was observed 38 times across 35 waterbodies or 21.6% of all surveyed

waterbodies (25.5%) compared to artificial ones (16.2%), this difference was not statistically

significant ($X^2 = 1.524$, p = 0.1085). However, we found significant variations in breeding

256 patterns across the different waterbody types ($X^2 = 368.8$, df = 9, p < 0.001) (Figure 4). In

257 artificial habitats, nearly all sites where breeding occurred were tanks, representing 28.1% of all 258 surveyed tanks (Figure 4). For natural habitats, stream pockets were the most common breeding 259 locations (15 waterbodies), with 50% of stream pockets having breeding occurrences (Figure 4). 260 Notably, 75% of all caves, both artificial and natural, showed breeding activity, although the 261 sample size for caves (four) was small (Figure 4). While artificial waterbodies maintained 262 relatively consistent breeding activity across the two breeding seasons (five and six breeding 263 occurrences), natural waterbodies showed a large decrease from 20 breeding to seven in the 264 subsequent season.

265 Breeding was observed for five of the ten species, with four species breeding in artificial 266 waterbodies and three species in natural waterbodies during the two survey periods (Figure 4). S. 267 salamandra exhibited the highest breeding frequency, with a total of eighteen events: four in 268 artificial habitats and 14 in natural ones (Figure 4). This species was found breeding in the 269 widest range of waterbody types, except fountains, where no species were found to breed (Figure 270 4). R. iberica had 15 breeding events, all within natural habitats, specifically in caves, streams, 271 and stream pockets (Figure 4). L. boscai had six breeding events, with five occurring in tanks 272 and one in a natural cave (Figure 4). P. perezi and T. marmoratus each had one breeding event, 273 both occurring in tanks (Figure 4).

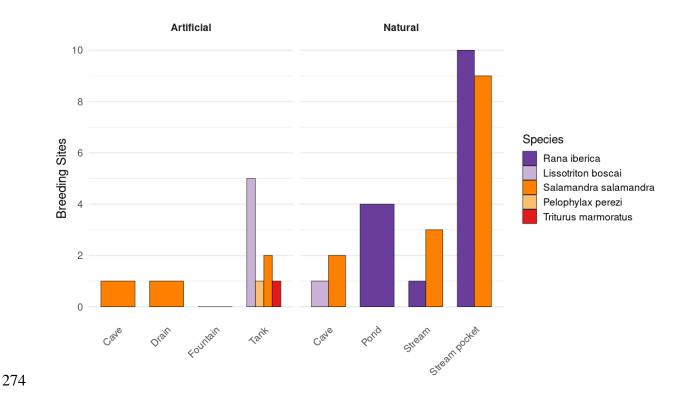


Figure 4: Number of breeding sites for amphibian species across different artificial and natural
waterbody types in Peneda National Park.

277 *Waterbodies characteristics*

278 The PCA biplot shows the relationships between key environmental variables, with the 279 first principal component (Dim1) explaining 31.2% and the second principal component (Dim2) 280 accounting for 20.5% of the variance, together capturing 51.7% of the total variation (Figure 5). 281 This analysis highlights clear distinctions between natural and artificial waterbodies, primarily 282 shaped by water flow and habitat characteristics. Natural habitats are located in the upper left of 283 the plot, closely associated with streams and ponds. Streams are linked to medium water flow, 284 indicating their connection to dynamic environments, while ponds are associated with still water 285 flow, reflecting stagnant conditions. Medium water flow and bare rock are positioned between 286 streams and drains, suggesting that drains share similarities with natural flowing water systems.

Although still water flow is positioned between ponds and tanks, indicating a shared characteristic of stagnation due to minimal water movement, tanks are distinctly located far to the right on the biplot. This positioning highlights their association with increased height above ground and greater water depth, highlighting their elevated and man-made nature, which further distinguishes them from natural waterbodies.

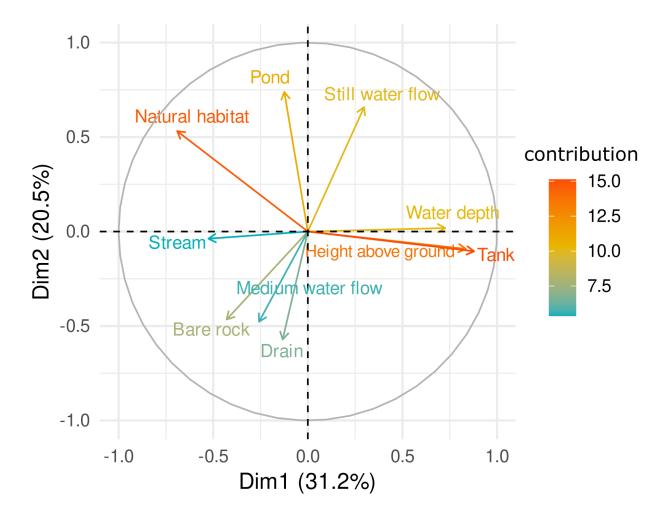




Figure 5. PCA biplot of environmental variables for waterbodies surveyed in Peneda National Park. The plot illustrates the contributions of environmental variables to the first two principal components (Dim1 and Dim2), which explain 31.2% and 20.5% of the variance, respectively. Arrows indicate the direction and strength of each variable's contribution and correlation to the components, with a color gradient showing higher contributions in warmer colors.

298 Discussion

299 Our study highlights the critical role that both natural and artificial habitats play in 300 supporting amphibian populations within the protected landscape of Peneda-Gerês National Park 301 (PNPG). These findings demonstrate that while natural waterbodies are critical for amphibians, 302 artificial aquatic features within villages in PNPG, particularly water tanks and drainage 303 channels, can play a crucial role in sustaining local amphibian populations, aligning with 304 previous studies on the importance of these artificial waterbodies in human-disturbed landscapes 305 (Knutson et al. 2004, Mazerolle 2005, Brand and Snodgrass 2010, Valdez et al. 2015, Yu et al. 306 2022, Caballero-Díaz et al. 2022, Romano et al. 2023). While natural waterbodies exhibited 307 higher overall species richness and occupancy rates, our results show that artificial waterbodies, 308 despite their human-altered nature, can serve as valuable complementary habitats for certain 309 amphibian species. Historical water tanks, in particular, not only supported a comparable 310 diversity of species as natural waterbodies but also showed significant occupancy rates and 311 breeding activity, particularly for species like the endemic L. boscai and T. marmoratus. This 312 suggests that these man-made water features are not merely supplementary but can play a crucial 313 role in providing essential breeding and refuge sites for these amphibian populations.

In total, we found ten amphibian species during the study, including six of the eight Anura and four of the five Urodela species that occupy the PNPG. *Rana iberica* was by far the most abundant among the observed species, constituting over 60% of all recorded individuals. This species was predominantly found in natural waterbodies, such as stream pockets and natural ponds. *L. boscai* and *S. salamandra* also showed high abundances, with the former being more abundant in artificial habitats, particularly tanks, and the latter more abundant in natural habitats. *T. marmoratus* was most abundant in artificial tanks, while *P. perezi* was notably more abundant 321 in natural ponds but also present in some tanks. These patterns align with previous studies in 322 PNPG, which identified these species as more common and widely distributed in the park 323 (Godinho et al. 1999, Soares et al. 2005). In contrast, B. spinosus and A. obstetricans were less 324 numerous but more frequently found in artificial environments. Meanwhile, D. galganoi, C. 325 *lusitanica*, and *E. calamita* were exclusive to natural habitats but recorded at only one or two 326 sites each, highlighting their small and fragmented distribution within the park (Soares et al. 327 2005). Although T. helveticus, P. cultripes, and H. arborea were not observed, this was likely 328 due to their scarcity in PNPG and preference for fossorial and arboreal habitats which were not 329 covered in this study (Soares et al. 2005).

330 We found that natural waterbodies had generally higher occupancy rates, abundance, and 331 breeding activity compared to artificial ones. Specifically, three-quarters of all natural 332 waterbodies were occupied at least once during the study, while half of the artificial sites were 333 occupied. Although slightly more amphibian species were observed breeding in natural habitats, 334 the difference in the total number of breeding events between natural and artificial sites was not 335 statistically significant, indicating that artificial waterbodies play a role in amphibian 336 reproduction. Comparable findings have shown that artificial sites, such as water tanks, ponds, 337 and drainage channels, can provide important breeding habitats for amphibians, especially in 338 landscapes altered by human activity (Brand and Snodgrass 2010, Ferreira and Beja 2013, 339 Caballero-Díaz et al. 2020, 2022, Romano et al. 2023). Among the natural habitats, stream 340 pockets were particularly crucial, with nearly all sites occupied at least once and serving as vital 341 refuges and breeding sites for species such as S. salamandra and R. iberica. However, although a 342 slightly greater number of amphibian species were observed breeding in natural habitats, the 343 difference in the number of breeding events between natural and artificial sites was not

statistically significant. We found that *R. iberica* bred exclusively in natural waterbodies, while *S. salamandra* highlighted its adaptability to different habitats by breeding in every type of
natural and artificial habitats, except fountains where no breeding occurred by any species. The
absence of breeding activity in fountains may be attributed to factors such as high water flow,
lack of suitable substrates, chemical cleaning, or frequent human disturbance, making them less
favorable for amphibian reproduction. While ponds and caves also supported some breeding
events they were much less commonly used in general.

351 Artificial waterbodies, despite lower overall occupancy rates, played a critical role in 352 supporting amphibian diversity and reproduction. Tanks were particularly important, with two-353 thirds being occupied at least once during the two seasons, the highest occupancy rate among the 354 artificial waterbodies. These historical tanks supported breeding activity for four out of the five 355 breeding species recorded (L. boscai, S. salamandra, T. marmoratus, and P. perezi), except for 356 *R. iberica*, which only bred in natural habitats. This diversity was greater than all natural 357 waterbodies combined, which supported only the three most common species (R. iberica, S. 358 salamandra, and L. boscai). Notably, T. marmoratus and P. perezi were found breeding 359 exclusively in tanks, albeit only once each. These results demonstrate how these historical 360 artificial waterbodies complement the park's natural waterbodies by providing vital refuges and 361 additional breeding sites for a diverse range of amphibian species. Typically elevated and fish-362 free, such tanks provide stable hydrological conditions and protection from predators, 363 significantly improving breeding success and larval survival (Garcia-Gonzalez and Garcia-364 Vazquez 2011, Ferreira and Beja 2013, Cabral et al. 2017, Arillo et al. 2022, Gould et al. 2024). 365 Additionally, consistent with previous studies (Mazerolle 2005, Yu et al. 2022), drainage

368 Although this study offers valuable insights into the role of human-modified waterbodies 369 for amphibian communities within the PNPG, it has several limitations. Surveys were conducted 370 over one week during just two consecutive breeding seasons, which may not capture the full 371 temporal dynamics and seasonal variations that could influence amphibian abundance, breeding 372 activity, and habitat preferences. Additionally, the study was limited to waterbodies that exist 373 within human-disturbed areas, such as villages and roads, within the northern part of the park. As 374 a result, the findings may not be fully representative of the amphibian communities and 375 waterbodies across the broader, more remote, and pristine areas of the national park. Lastly, 376 another limitation is the potential for detection biases. While we conducted extensive surveys, some species, especially those with cryptic behaviors or low populations, may have been 377 378 overlooked, resulting in an incomplete representation of the community's true biodiversity. 379 Future research should aim to address these limitations by expanding the geographic and 380 temporal coverage, incorporating a wider range of waterbodies, and incorporating more 381 comprehensive survey techniques to provide a better understanding of amphibian diversity and 382 conservation in PNPG.

Looking ahead, the integration of artificial waterbodies into broader conservation strategies will be essential for sustaining amphibian populations not only within PNPG but also in the face of global challenges such as climate change and habitat loss (Briggs 2010, Brand and Snodgrass 2010, Garcia-Gonzalez and Garcia-Vazquez 2011). While artificial waterbodies, such as historic water tanks and drainage channels, typically have lower species richness compared to

388 natural ones, they often serve as the only viable breeding and refuge sites in human-altered 389 landscapes (Brand and Snodgrass 2010, Plăiasu et al. 2012, Buono et al. 2019, Valdez et al. 390 2021). As climate change intensifies, leading to more frequent and severe droughts in the Iberian 391 Peninsula (Soares et al. 2023, Alvarez et al. 2024), artificial waterbodies will become 392 increasingly crucial in sustaining biodiversity. Such waterbodies will be important to support not 393 only species like *T. marmoratus*, which are highly vulnerable to climate-induced range 394 contractions (Préau et al. 2022) but also serve as essential refuges for all species as natural 395 habitats continue to diminish and their availability becomes more unpredictable. Indeed, our 396 study found that while breeding events in artificial waterbodies remained stable, natural 397 waterbodies saw a dramatic two-thirds decline during the second breeding season, underscoring 398 the crucial role of artificial waterbodies as reliable refuges amid fluctuating conditions. This 399 hydrological stability is particularly important for species like newts, which are more sensitive to 400 habitat changes and climate impacts, potentially reducing the persistence of all but a few mobile 401 and opportunistic species (Ficetola and De Bernardi 2004).

402 To maximize the benefits of artificial waterbodies, it's crucial to integrate their 403 management with natural ecosystems, especially in areas like PNPG where land abandonment 404 has led to the deterioration of these structures. Similar impacts have been observed in northern 405 Italy, where land abandonment has negatively impacted the breeding sites of endangered 406 amphibians (Canessa et al. 2013, Arillo et al. 2022). While fountains are more likely to be 407 preserved for their historical and aesthetic value, they offer little support for amphibian 408 conservation. In contrast, water tanks, which provide crucial breeding habitats for several 409 amphibian species, receive less conservation attention due to their lower cultural significance 410 and are more vulnerable to neglect as traditional village life and agricultural practices decline.

411 Effective management must not only conserve these structures but also address threats such as 412 physical deterioration from land abandonment, chemical cleaning, and the introduction of non-413 native species (Chiacchio et al. 2024). Incorporating traditional and environmentally friendly 414 land management practices can help maintain a mosaic of natural and human-altered landscapes 415 that support optimal amphibian habitats (Briggs 2010, Plăiasu et al. 2012). Additionally, 416 incorporating key habitat characteristics, such as ramps in artificial habitats, has also proven 417 effective in enhancing amphibian conservation efforts (Yu et al. 2022, Arillo et al. 2022). By 418 integrating artificial waterbody management with natural ecosystems and holistic conservation 419 practices, we can ensure these habitats remain critical refuges for amphibians, preserving both 420 cultural heritage and local biodiversity amid ongoing environmental challenges.

421 Conclusion

422 This study underscores the often-overlooked vet crucial role of artificial habitats in 423 sustaining amphibian populations within Peneda-Gerês National Park. Centuries-old water tanks 424 and irrigation channels, remnants of traditional village life, not only offer essential refuges and 425 breeding sites for amphibians but also reflect the region's rich cultural heritage. As natural 426 wetlands decrease and environmental conditions grow more unpredictable, especially with the 427 rising frequency and intensity of droughts in the Iberian Peninsula, many of these man-made 428 structures will become increasingly critical for maintaining local biodiversity. By integrating the 429 management of artificial habitats with broader conservation strategies, we can better support 430 amphibian populations, improve ecological resilience, and preserve the cultural landscapes that 431 are intrinsic to the region's heritage.

432 Acknowledgments

The authors would like to thank the Peneda-Gerês National Park, as well as the Institute	
for Nature Conservation and Forests, Portugal (ICNF) for the opportunity to conduct field	
research within the Park. We also acknowledge the support of the German Centre for Integrative	
Biodiversity Research (iDiv) Halle-Jena-Leipzig, funded by the German Research Foundation	
(DFG-FZT 118, 202548816). Open access funding enabled and organized by Projekt DEAL.	
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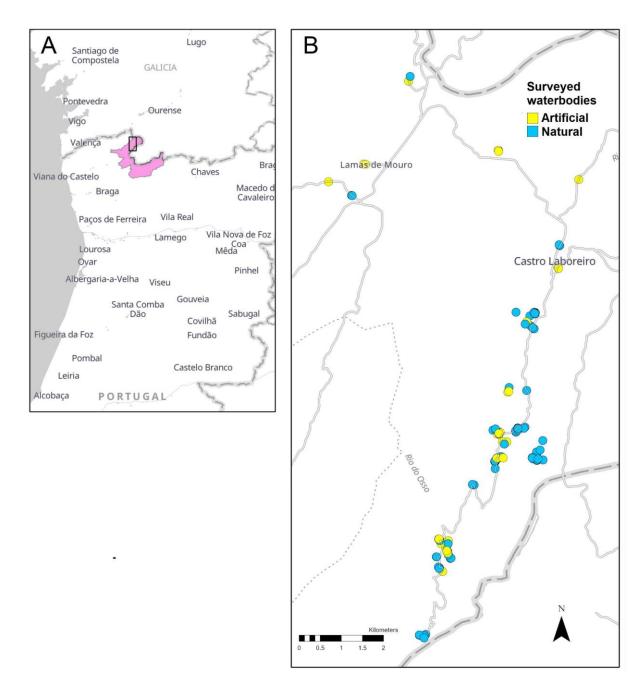
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599 Appendix S1



600

601 **Figure S1.** (A) Location of Peneda-Gerês National Park in Portugal (highlighted in pink). (B)

602 Surveyed artificial (yellow) and natural (blue) waterbodies within the park.

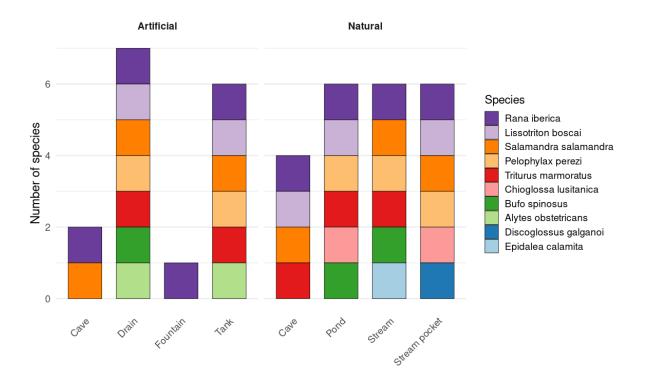




Figure S2. Stacked bar plot depicting amphibian species richness in artificial and natural waterbody types. Each bar represents the number of species for each type of waterbody, with colors indicating the different species observed within that specific waterbody type. Species are ordered by the most commonly found.

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